

- b) in the parent lipolytic enzyme, selecting at least one amino acid residue which comprises at least one atom within 10 Å of the C atom at the sn2 position of the glycerol part of a substrate triglyceride in a three-dimensional structure of the parent lipolytic enzyme and the substrate,
- c) making alterations of the at least one amino acid residue,
- d) preparing the variant resulting from steps a-c,
- e) testing substrate specificity of the variant,
- f) selecting a variant having an altered substrate specificity, and
- g) producing the selected variant.

66. The method of claim 65, wherein the parent lipolytic enzyme is a lipolytic enzyme from the *Humicola* family or the *Zygomycetes* family.

67. A method of producing a lipolytic enzyme variant comprising:

- a) selecting a parent lipolytic enzyme from the *Humicola* family or the *Zygomycetes* family,
- b) selecting at least one amino acid residue corresponding to any of amino acids 20-25, 56-64, 81-85 and 255-269 in the *Humicola lanuginosa* lipase
- c) making alterations of the at least one amino acid residue,
- d) preparing the variant resulting from steps a-c,
- e) testing substrate specificity of the variant, and
- f) selecting a variant having an altered substrate specificity.

68. The method of claim 65, wherein the parent lipolytic enzyme is the lipase of *Humicola lanuginosa* strain DSM 4109.

69. The method of claim 65, wherein the altered substrate specificity is a lower ratio of activity towards a C₄-C₈ acyl bond in a triglyceride and a C₁₆-C₂₀ acyl bond in a triglyceride.

70. The method of claim 65, wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, and the selected amino acid residues comprise an amino acid corresponding to Y21, E56, D57, V60, G61, D62, R81, S83, R84, L259, Y261 or G266 in the *Humicola lanuginosa* lipase.

71. The method of claim 65, wherein the altered substrate specificity is a lower ratio of activity towards a C₁₆-C₂₀ acyl bond in a triglyceride and a C₄-C₈ acyl bond in a triglyceride.

72. The method of claim 65, wherein the altered substrate specificity is a higher phospholipase activity.

73. The method of claim 72, wherein the parent lipolytic enzyme has a phospholipase activity below 50 PHLU/mg and/or a ratio of phospholipase activity to lipase activity below 0,1 PHLU/LU.

74. The method of claim 65, wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, and the selected amino acid residues comprise an amino acid corresponding to R81, R84, S85, or any amino acid within the range 263-267 in the *Humicola lanuginosa* lipase.

75. The method of any of claims 65, wherein the alterations comprise an insertion of a peptide extension at the C-terminal.

76. The method of any of claims 65, wherein the alterations comprise an insertion of a peptide extension at the C-terminal comprising from 1 to 5 amino acid residues.

77. The method of any of claims 65, wherein the altered activity is a higher hydrolytic activity on a digalactosyl-diglyceride.

78. The method of claim 65, wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, and the selected amino acid residues comprise an amino acid corresponding to 21, 23, 26, 57, 62, 81, 83, 84, 85, 266, 267 or 269 in the *Humicola lanuginosa* lipase.

79. An isolated lipolytic enzyme which:

- a) has an amino acid sequence that is at least 80 % homologous with the amino acid sequence of a reference lipolytic enzyme of the *Humicola* family or the *Zygomycetes* family;
- b) has an amino acid sequence that differs from the amino acid sequence of the reference lipolytic enzyme wherein the difference comprises:

- i) an amino acid substitution, deletion or insertion at a position corresponding to A20, Y21, G23, K24, N25, V63, R81, G82, R84, A257, W260, Y261, F262 or G266 in the *Humicola lanuginosa* DSM 4109 lipase;
- ii) a substitution of an amino acid corresponding to C268 or L269 in the *Humicola lanuginosa* DSM 4109 lipase;
- iii) a substitution corresponding to V60G, D62E, L93K, L97Q, K98E,F, E99D, P256A, G263E,Q,R,F,N, L264A,C,P,F,G,V,I, I265L,N,F or T267A,Q,P,S,V,E in the *Humicola lanuginosa* DSM 4109 lipase;
- iv) an insertion corresponding to T267GS or T267GL in the *Humicola lanuginosa* DSM 4109 lipase;
- v) a peptide extension at the C-terminal which is A, P, MD, CP, AG, DG, AGG, PVGF, AGRF, PRGF, AGGF or AGGFS;
- vi) a peptide extension at the C-terminal of 40-50 amino acids; or
- vii) a truncation of 1, 2, 3, 4, 5 or 6 amino acids at the C-terminal.

80. The lipolytic enzyme of claim 79, wherein lipolytic enzyme has an amino acid substitution corresponding to R84K,L,W, W260H,Q,C, G266A,C,D,N,L,I,S,T,P,V,F,W,E,K,R,Y or L269N,I,S in the *Humicola lanuginosa* DSM 4109 lipase .

81. The lipolytic enzyme of claim 79, which further comprises at least one amino acid substitution, a deletion or an insertion corresponding to any of positions 22, 56-59, 61, 64, 83, 85, 91, 94, 249, 255 or 259.

82. The lipolytic enzyme of claim 79, which further comprises at least one amino acid substitution selected from the group consisting of: S83T, G91A, N94D, D96S,W,F,G, Q249R and L259N,R,S,M,Q.

83. The lipolytic enzyme of 79, which further comprises at least one amino acid substitution selected from the group consisting of D62A,G,V, K98D, E99K, P256T, G263A or I265T,G,V.

84. The lipolytic enzyme of any of claim 79, which further comprises a peptide extension at the N-terminal compared to the reference lipolytic enzyme.

85. The lipolytic enzyme of any of claim 79, wherein the reference lipolytic enzyme is a lipase from *Humicola lanuginosa*.

86. The lipolytic enzyme of any of claim 79, wherein the reference lipolytic enzyme is alipase from *Rhizomucor miehei*.

87. The lipolytic enzyme of any of claim 79, wherein the reference lipolytic enzyme is a lipase from *Fusarium oxysporum*.

88. The lipolytic enzyme of any of claims 79, which comprises an amino acid alteration at a position corresponding to Y21, E56, D57, V60, G61, D62, R81, S83, R84, L259, Y261 or G266 in the *Humicola lanuginosa* lipase.

89. The lipolytic enzyme of any of claim 79, which comprises an amino acid alteration at a position corresponding to R81, R84, S85, G263, L264, I265, G266, T267 or L269 in the *Humicola lanuginosa* lipase.

90. The lipolytic enzyme of any of claim 79, which comprises an amino acid substitution corresponding to G263A,E,Q,R; L264A,C,P,Q; I265L,N,T; G266A, C, D, N, L, I, S, T, P, V or T267A,Q or L269N in the *Humicola lanuginosa* lipase.

91. The lipolytic enzyme of any of claim 79, which comprises a deletion of amino acid residues at positions corresponding to positions C268 and L269 in the lipase of *Humicola lanuginosa* strain DSM 4109.

92. The lipolytic enzyme of claim 79, which comprises an alteration in the lid of the lipolytic enzyme which is a substitution of a negatively charged amino acid residue in the lid of the lipolytic enzyme with a neutral or positively charged amino acid residue, or a substitution of a neutral amino acid residue in the lid of the lipolytic with a positively charged amino residue.

93. The lipolytic enzyme of claim 79, which comprises an alteration in the lid of the lipolytic enzyme at a position corresponding to position G91, D96 or E99 in the *Humicola lanuginosa* lipase.
